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## Cellular Receptors for Advanced Glycation End Products

### Implications for Induction of Oxidant Stress and Cellular Dysfunction in the Pathogenesis of Vascular Lesions

Ann Marie Schmidt, Osamu Hori, Jerold Brett, Shi Du Yan, Jean-Luc Wautier, David Stern

**Abstract** Advanced glycation end products (AGEs) form by the interaction of aldoses with proteins and the subsequent molecular rearrangements of the covalently linked sugars, eventuating in a diverse group of fluorescent compounds of yellow-brown color. This heterogeneous class of nonenzymatically glycated proteins or lipids is found in the plasma and accumulates in the vessel wall and tissues even in normal aging. As a consequence of hyperglycemia, AGE formation and deposition are much enhanced in diabetes, in which their presence has been linked to secondary complications, especially microvascular disease. This review summarizes the cellular interactions of AGEs and describes the central role of a novel receptor for AGE (RAGE). RAGE, an immunoglobulin superfamily member, mediates the binding of AGEs to endothelial cells and mononuclear phagocytes, interacts with a lactoferrin-like polypeptide that also binds AGEs, and appears to activate intracellular signal transduction mechanisms con-

sequent to its interaction with the glycated ligand. RAGE is expressed by ECs, mononuclear phagocytes, smooth muscle cells, mesangial cells, and neurons, indicating a potential role in the regulation of their properties in homeostasis and/or their dysfunction in the development of diabetic complications. Since AGEs have been shown to generate reactive oxygen intermediates, tethering of AGEs to the cell surface by their receptors focuses oxidant stress on cellular targets, resulting in changes in gene expression and the cellular phenotype. The discovery of RAGE and development of reagents to block its interaction with AGEs should provide insights into the role of this ligand-receptor interaction in the pathogenesis of diabetic complications and, potentially, atherosclerosis. (*Arterioscler Thromb.* 1994;14:1521-1528.)

**Key Words** • endothelial cells • diabetes • monocytes • glycation • atherosclerosis

When proteins or lipids are exposed to aldose sugars, they undergo nonenzymatic glycation and oxidation.<sup>1-7</sup> Initially, this leads to the formation of early glycation products, Schiff bases, and Amadori products, the best known of which is hemoglobin A<sub>1c</sub> for its use in monitoring glucose control over days or weeks in patients with diabetes.<sup>8</sup> The early glycation products are still in equilibrium with plasma glucose, and when glucose levels fall, the early glycation products can dissociate to the native proteins.<sup>4</sup> Alternatively, if glycation continues, further largely undefined molecular rearrangements occur, resulting in formation of the irreversible advanced glycation end products (AGEs). The latter constitute a heterogeneous class of structures that are yellow-brown pigments, fluoresce, tend to form cross-links, generate reactive oxygen intermediates, and recognize a class of receptors on cellular surfaces.<sup>1,7,9-13</sup> Certain AGEs have been characterized in detail, including pentosidine and N<sup>ε</sup>-(carboxymethyl)lysine.<sup>14-17</sup>

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To detect AGEs in vivo, several groups have developed polyclonal and monoclonal antibodies selective for AGE-modified forms of proteins<sup>18-21</sup>; we have developed such an immunologic reagent by immunizing animals with AGE-modified keyhole limpet hemocyanin and affinity-purifying the IgG population on an AGE albumin column.<sup>21</sup> The affinity-purified anti-AGE IgG demonstrates the presence of AGEs in the plasma and in the tissues of patients with diabetes.<sup>21</sup> For example, in a case of diffuse diabetic glomerulosclerosis, immunostaining shows extensive deposition of AGEs (Fig 1A); staining of the vessel wall and Bowman's capsule is demonstrated. In contrast, there is no immunostaining in a sample from normal kidney (data not shown) and on sections from the same individual as in Fig 1A stained with nonimmune IgG (Fig 1B). Kidney from a spectrum of patients with diabetes demonstrates variable extents of AGE deposition, although AGE accumulation is always increased compared with age-matched nondiabetic subjects. AGEs have also been identified in the vessel wall and skin from patients with diabetes.<sup>22-25</sup> These results, taken together with biochemical analyses of tissues for pentosidine and carboxymethyl lysine,<sup>16,22-25</sup> are consistent with the hypothesis that formation of AGEs correlates with the development of diabetic complications.<sup>1,2,4</sup> Previous studies have emphasized a possible link between accumulation of AGEs in the vessel wall and the accelerated vascular disease that occurs during the course of diabetes.<sup>1,2,4</sup> This is consistent with the identification of AGEs

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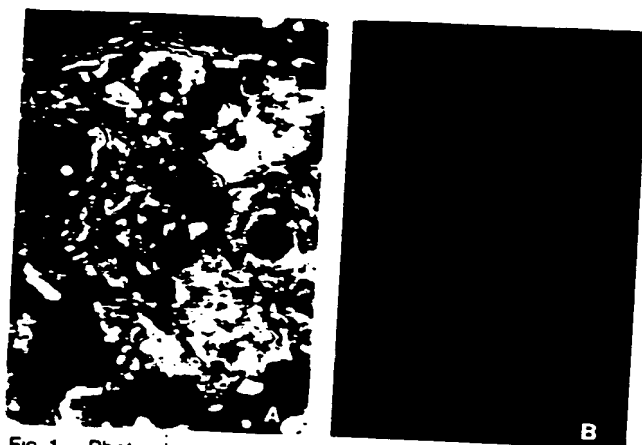


FIG 1. Photomicrographs showing immunostaining of kidney from a 20-year-old patient with diffuse diabetic glomerulosclerosis with anti-advanced glycation end product IgG (A) and nonimmune control IgG (B). Magnification  $\times 300$ .

in the vessel wall of some patients with atherosclerosis and diabetes.<sup>25</sup>

AGEs also form on long-lived proteins during normal aging<sup>1,2,4</sup> and in abnormal deposits of protein of long duration; a striking example occurs in Alzheimer's disease,<sup>26-28</sup> in which the neurofibrillary tangles characteristic of intraneuronal Alzheimer's disease lesions are seen to be glycosylated, most probably the tau that constitutes the paired helical filaments.<sup>28</sup> These data emphasize the potential contribution of AGEs to a range of disorders even beyond diabetes. Another well-described example of this is the presence of AGEs in hemodialysis-associated amyloidosis, a common complication observed in patients on long-term hemodialysis.<sup>29</sup> In this case, it is likely that AGEs are implicated in the pathogenesis of the inflammatory arthropathy observed in this disorder.

The close association of AGEs with cellular elements, as depicted in the photomicrograph of diabetic renal tissue (Fig 1A), indicated the importance of characterizing the molecular basis and pathological consequences of such interactions.

### Characterization of Cellular Receptors for AGEs

AGE-modified ligands, both those prepared *in vitro* and those obtained *in vivo*, have been used to elucidate their cellular associations, initially with cultured mononuclear phagocytes (MPs) and later with endothelial cells (ECs). The results demonstrated specific and saturable surface binding (with dissociation constants in the range of 50 to 200 nmol/L) as well as cellular uptake by endocytosis.<sup>4,12,20,30</sup> Since EC binding sites for AGEs were trypsin sensitive and elutable in the presence of detergent, we suspected that they were composed of polypeptide(s) and undertook their isolation by a series of chromatographic steps (hydroxylapatite, fast protein liquid chromatography [FPLC] Mono S, and FPLC gel filtration). We isolated polypeptides from bovine lung tissue on the basis of their ability to bind AGE ligands<sup>12</sup>; one was named receptor for AGE (RAGE), and the other is a lactoferrin-like polypeptide (LF-L).

Molecular cloning studies have shown RAGE to be a newly identified member of the immunoglobulin superfamily of cell-surface molecules.<sup>13</sup> The human, murine, rat, and bovine counterparts show great similarity according to analysis of the respective cDNAs (Reference 13 and unpublished observations). The hydropathy plot of the bovine cDNA demonstrates the presence of three putative immunoglobulin-like domains in the extracellular region: one V-type domain followed by two C-type domains. This region also includes two putative *N*-linked glycosylation sites and placement of cysteine residues at sites expected to stabilize immunoglobulin-like loops. Following the extracellular domain, RAGE displays a single putative transmembrane-spanning region and a short, highly charged cytosolic tail. The latter domain shows greatest homology to the B-cell activation marker CD20.<sup>13</sup> Transfection of RAGE cDNA into 293 cells resulted in both the expression of the protein and specific binding of radioiodinated AGE albumin, which could be blocked by anti-RAGE IgG. These data indicate that RAGE is an integral membrane protein capable of serving as a cell-surface acceptor site for AGEs.

At the outset of our studies to characterize cellular AGE receptors, we expected to identify polypeptide(s) closely resembling the recently described scavenger receptors composed of trimeric, collagen-like molecules that bind acetylated low-density lipoprotein.<sup>31</sup> The identification of RAGE as a member of the immunoglobulin superfamily suggested that in addition to interacting with AGEs, it might serve other functions, including mediation of cell-cell recognition or binding of growth factors or cytokines. The presence of multiple ligands for immunoglobulin superfamily receptors, such as intercellular adhesion molecule-1, is well known.<sup>32</sup> This has led us to search for non-AGE ligands that show a high-affinity interaction with RAGE; two such candidate polypeptides have been identified, and their characterization should provide new insights into the role of RAGE in cell spreading on physiological matrices and its capacity to function as a recognition site for a novel mediator (A.M.S. et al, unpublished data). With respect to the short cytosolic tail of RAGE, we hypothesize that it probably binds to intracellular signal transduction molecules, and studies to test this concept are currently under way.

The other polypeptide isolated from bovine lung tissue on the basis of its capacity to bind AGEs was LF-L.<sup>12</sup> It displayed characteristics closely resembling milk-derived lactoferrin: (1) the amino terminal sequences were virtually identical, (2) antibodies to lactoferrin reacted with LF-L and vice versa, and (3) milk-derived lactoferrin bound [<sup>125</sup>I]-AGE albumin in a manner comparable to LF-L isolated from lung (this occurs in the absence of RAGE<sup>12</sup>). Although milk-derived lactoferrin has an  $M_r \approx 80$  kD, similar to the material isolated from bovine lung, immunoblotting of ECs and MPs has shown a lower-molecular-weight form of LF-L/lactoferrin with an  $M_r$  closer to  $\approx 30$  kD,<sup>12,20,33</sup> which is likely to result from proteolytic processing. LF-L binds noncovalently and with high affinity to the extracellular domain of RAGE, and the resulting complex also binds AGEs.<sup>33</sup> On ECs and MPs, the receptor appears to be present as an RAGE/LF-L complex<sup>12,20</sup>; cross-linking studies demonstrate a higher-molecular-weight band containing both RAGE and LF-L immu-

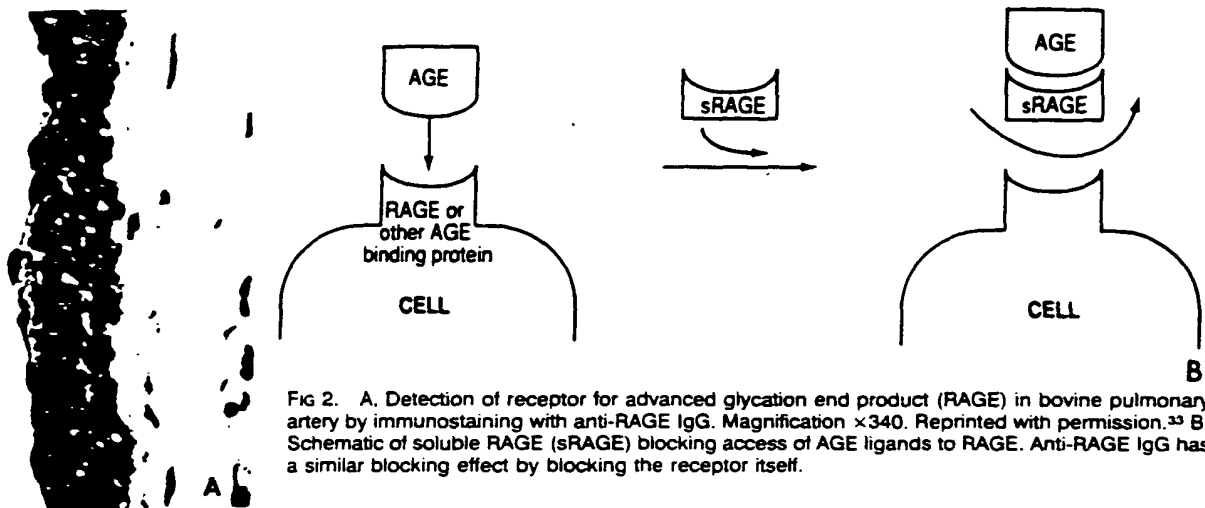


Fig 2. A, Detection of receptor for advanced glycation end product (RAGE) in bovine pulmonary artery by immunostaining with anti-RAGE IgG. Magnification  $\times 340$ . Reprinted with permission.<sup>33</sup> B, Schematic of soluble RAGE (sRAGE) blocking access of AGE ligands to RAGE. Anti-RAGE IgG has a similar blocking effect by blocking the receptor itself.

noreactivity, and radioligand binding studies with AGE albumin show that antibody to either RAGE or LF-L (these antibodies are not cross-reactive) blocks specific binding of AGE ligands to the cell surface. The site for cellular assembly of RAGE/LF-L complex and its functional significance versus RAGE alone has not been defined at this time. It is possible, however, that the presence of both molecules increases the repertoire of AGE-like structures recognized by the cell surface, thereby enhancing cellular interactions.

The existence of other cellular binding sites for AGEs has also been postulated<sup>11</sup>; however, these polypeptides have not been further characterized, and thus, their potential relation to RAGE and LF-L is unclear.

#### Endothelial Cells

Since ECs form the vascular interface, endothelium is exposed to AGEs in the circulation, both AGE-modified plasma proteins and AGEs that formed on cellular surfaces such as diabetic red cells (RBCs; see below), as well as those present in the underlying subendothelial matrix. This consideration emphasized the potential importance of ECs in the processing/clearance of AGEs, in addition to the possible effects of AGEs as perturbants of EC properties. In the latter context, we have shown that exposure of cultured ECs to AGE albumin suppressed expression of the anticoagulant cofactor thrombomodulin, increased diffusional transit of macromolecular solutes across the EC monolayer, and led to sustained induction of low levels of the procoagulant cofactor tissue factor (in addition, AGEs enhanced the EC procoagulant response to tumor necrosis factor<sup>30</sup>). By use of monospecific antibody and oligonucleotide riboprobes, RAGE antigen and mRNA have been identified in ECs both *in vivo* and *in vitro*.<sup>34</sup> Immunostaining of a bovine pulmonary artery (Fig 2A) demonstrates RAGE antigen in the endothelium and vivid staining of vascular smooth muscle. RAGE is observed in the microvasculature and macrovasculature, although in some normal vascular beds its expression appears to be at low levels. Expression of RAGE in smooth muscle was a consistent finding in our tissue survey<sup>34</sup> and has been confirmed by *in situ* hybridization and by studies with cultured vascular smooth muscle.<sup>34</sup>

The functional significance of RAGE in smooth muscle cells has not been defined, although the above results showing high levels of RAGE in smooth muscle suggest that AGE-RAGE interaction is likely to have an effect on cellular properties.

Endothelial RAGE has a central role in mediating vascular interactions of AGEs in the intravascular space. When AGE albumin is infused intravenously into mice, it displays a rapid initial phase of plasma clearance not observed with native albumin and accumulates principally in the liver, lung, kidney, intestine, and heart.<sup>35</sup> Experiments with AGE albumin linked to colloidal gold particles have shown that AGE albumin-colloidal gold conjugates infused into murine coronary vasculature become closely associated with the endothelium. After surface binding, AGEs are endocytosed, and a portion of the ligand traverses the EC layer by transcytosis, being released at the albuminal surface. RAGE expressed on the endothelium thus provides a pathway for receptor-mediated uptake of the ligand from the intravascular space with the potential for ultimate delivery of the glycosylated proteins to the subendothelium, where AGEs can cross-link with long-lived extracellular matrix components. Both the rapid phase of AGE albumin plasma clearance and its deposition in the tissues are virtually completely blocked by anti-RAGE IgG or by administration of soluble RAGE (sRAGE; the latter is a form of RAGE composed of the extracellular domain and is obtained from bovine lung, probably as a result of proteolysis during the preparation procedure or by expression of the recombinant protein<sup>12,13</sup>). The likelihood that these early interactions of circulating AGEs with RAGE set in motion events with more far-ranging functional consequences is suggested by our observation that within an hour of the AGE albumin infusion, levels of interleukin-6 mRNA rise and activation of the transcription factor NF- $\kappa$ B occurs, each of which is blocked by administration of anti-RAGE IgG but not by similar concentrations of nonimmune IgG.<sup>21,35</sup>

These data are consistent with the hypothesis that RAGE expressed by endothelium interacts readily with AGEs in the intravascular space and that anti-RAGE

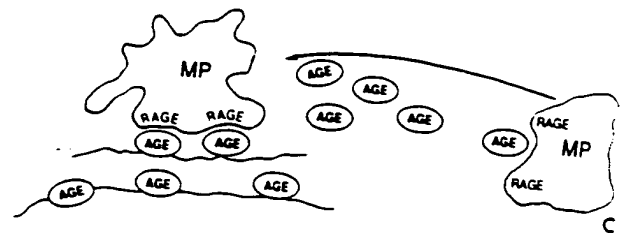
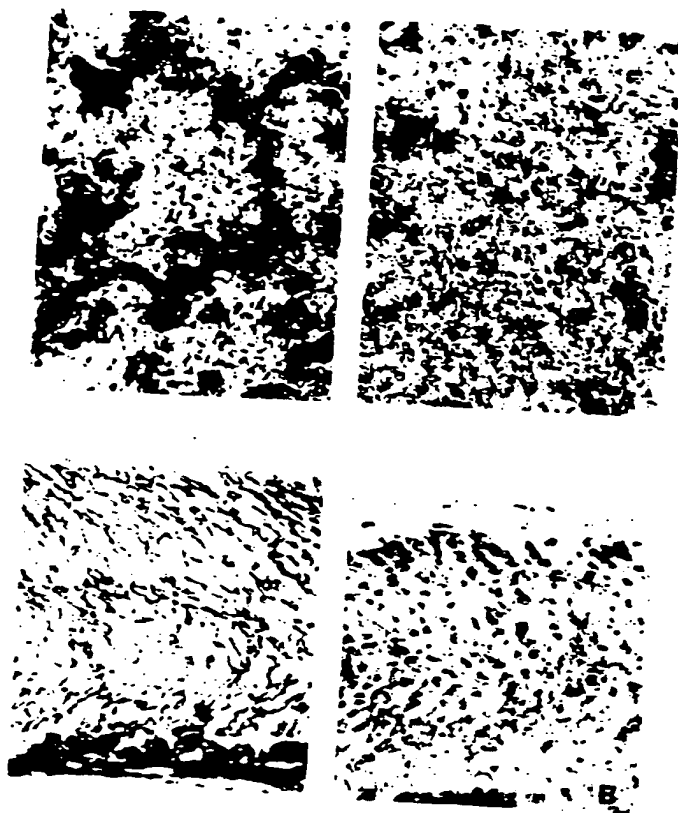


Fig 3. A, Phagokinetic track assay: mononuclear phagocyte (MP) migration on matrix composed of native albumin or advanced glycation end product (AGE) albumin. MPs were allowed to migrate for 6 hours on matrices of native albumin (left) or AGE albumin (right) that had been coated with colloidal gold particles. The dark tracks left by locomoting MPs are visualized by dark-field microscopy. Magnification  $\times 125$ . Reprinted with permission.<sup>20</sup> B, Implantation of polytetrafluoroethylene (PTFE) tubes with adsorbed AGE albumin into rats. PTFE tubes impregnated with either native albumin (left) or AGE albumin (right) were implanted into the subcutaneous tissue of rats. The tubes were removed after 4 days and examined for the presence of infiltrating cells by hematoxylin and eosin staining. Magnification  $\times 110$ . Reprinted with permission.<sup>20</sup> C, Schematic of MP interaction with soluble AGEs, which induce MP chemotaxis, and immobilized AGEs (as in extracellular matrix), which prevent MP migration. RAGE indicates receptor for AGE.

IgG and sRAGE are potentially useful reagents to dissect the contribution of AGE-RAGE interaction in complex *in vivo* settings (Fig 2B). They function by blocking access of receptor to the ligand (the antibody does not appear to result in cellular activation similar to that observed consequent to the binding of AGEs; see below).

### Mononuclear Phagocytes

As professional scavengers, MPs would be expected to interact with AGEs, potentially resulting in their clearance and degradation. Among the first studies concerning the interaction of AGEs with cellular elements were experiments demonstrating the presence of specific cell-surface binding sites for AGEs on MPs.<sup>4</sup> Interaction of AGEs with MPs has been shown to induce an activated phenotype, manifested by induction of platelet-derived growth factor, insulin-like growth factor-1, and proinflammatory cytokines such as interleukin-1 and tumor necrosis factor.<sup>36-38</sup> By analogy with ECs, our recent studies have shown that AGE-monocyte interaction is mediated by RAGE and LF-L.<sup>20</sup>

One way in which MPs gain access to AGE-rich microenvironments could result from AGE-mediated induction of monocyte chemotaxis.<sup>20</sup> Soluble AGE ligands, such as AGE albumin prepared *in vitro* or AGEs immunisolated from diabetic plasma,<sup>20</sup> induce migration of MPs studied in a modified chemotaxis chamber (similar results have been observed with AGE- $\beta_2$ -microglobulin isolated from patients with hemodialysis-associated amyloidosis<sup>39</sup>). This migratory response is blocked by anti-RAGE IgG or sRAGE, although the latter reagents have no effect on MP migration in

response to formylated chemotactic peptides.<sup>20</sup> In contrast to the effect of soluble AGEs, when the upper surface of the chemotactic chamber membrane is coated with AGE ligand, monocyte migration to the lower compartment in response to another signal, such as fMet-Leu-Phe, is blocked.<sup>20</sup> These observations suggest that immobilized AGEs, such as those found in basement membranes, have the capacity to slow MP migration. This haptotactic response of MPs to immobilized AGEs is also blocked by anti-RAGE IgG/F(ab')<sub>2</sub> or sRAGE. Consistent with these data, experiments using the phagokinetic track assay of Albrecht-Buehler<sup>40</sup> have shown that MPs plated on native matrices show long paths of migration (Fig 3A, left), whereas on AGE-modified matrices their paths are much shorter (Fig 3A, right). To apply these observations in cell culture to the *in vivo* setting, AGE albumin was adsorbed to polytetrafluoroethylene (PTFE) mesh and was implanted subcutaneously into rats. PTFE mesh impregnated with AGEs provides a vehicle for sustained slow release of AGEs from the mesh, with a large reservoir of AGE albumin still retained on the mesh. Intense infiltration of interstices of the PTFE with adsorbed AGEs by MPs was observed (Fig 3B, right), and some of these cells had undergone epithelioid changes, suggesting that activation had occurred. In contrast, PTFE similarly impregnated with native albumin showed little cellular infiltration: the host response, typically a foreign-body reaction, was largely confined to the graft-tissue interface (Fig 3B, left). These data suggest a hypothetical scheme for considering the effect of AGEs on MPs (Fig 3C): soluble AGEs induce monocyte migration, with the cells migrating down a concentration gradient. When

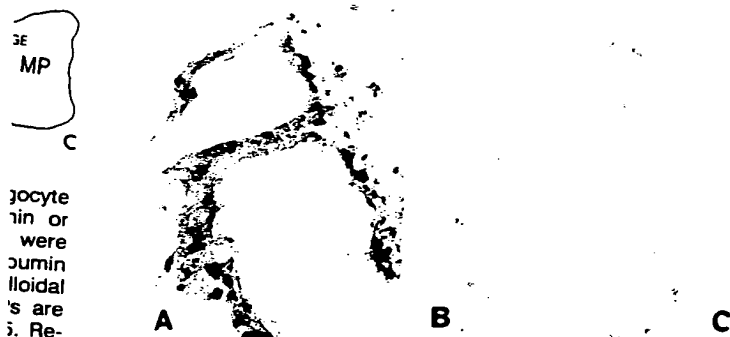


FIG 4. Immunohistological detection of malondialdehyde determinants in the vasculature of rats infused with advanced glycation end product (AGE) albumin vs native albumin. Rats were infused with AGE albumin (1 mg per animal; A) or native albumin (1 mg per animal; B); after 60 minutes, they were sacrificed and tissues were processed for detection of malondialdehyde epitopes by indirect immuno-alkaline phosphatase. C, Sections from an animal infused with AGE albumin were reacted with nonimmune IgG and then processed as in A and B. Magnification  $\times 400$ . Reprinted with permission.<sup>21</sup>

MPs reach a site of immobilized AGEs in the tissues or vessel wall, their migration (in response to either AGEs or another stimulus) is diminished, allowing them to bind to the AGE-modified surface and become activated. This could provide a mechanism for attracting and retaining MPs at sites of AGE deposition in the tissues, potentially contributing to the development of tissue lesions after AGE-induced monocyte migration. These observations concerning the effects of AGEs on MP motility are reminiscent of studies concerning induction and arrest of MP chemotaxis by oxidized and minimally modified low-density lipoprotein.<sup>41,42</sup>

#### Other Cell Types

A survey of normal tissues for RAGE shows that it is present in mesangial cells and some neurons.<sup>34</sup> RAGE is expressed in an as yet unidentified population of cortical neurons; experiments with embryonic rat cortical neurons have suggested a possible role in cell spreading on matrices (A.M.S. et al, unpublished data). In addition, spinal motor neurons express high amounts of RAGE antigen and mRNA, as do neurons in the peripheral and autonomic nervous systems. The presence of RAGE in neurons and in smooth muscle (see above) suggests that RAGE has functions beyond its interaction with AGEs. We hypothesize that the non-AGE ligands of RAGE may have a role in homeostasis distinct from the perturbing effects of AGEs; ie, the function of RAGE can hardly be solely to scavenge AGEs.

#### Induction of Oxidant Stress

Previous studies have linked glycation and oxidation and have shown that nonenzymatically glycated proteins can generate reactive oxygen intermediates<sup>9,10</sup>; the retention of such AGEs in close approximation to the cell membrane (via binding to RAGE or RAGE/LF-L) and their subsequent internalization would appear to create a likely environment for the development of oxidant stress. In the extracellular space, generation of reactive oxygen intermediates by deposits of glycated proteins can have important consequences and is one likely explanation for the observed ability of AGEs to quench nitric oxide.<sup>43</sup> In the context of the cellular interactions of AGEs, induction of oxidant stress results in modulation of a range of homeostatic mechanisms. Incubation of cultured ECs with AGE albumin or AGEs immunisolated from diabetic plasma led to these evidences

of oxidant stress<sup>21</sup>: (1) generation of thiobarbituric acid-reactive substances, (2) induction of heme oxygenase mRNA, and (3) activation of the transcription factor NF- $\kappa$ B. Enhanced expression of heme oxygenase type 1 and nuclear translocation of NF- $\kappa$ B have been linked to oxidant stress by previous studies with other oxidizing stimuli.<sup>44,45</sup> Each of these AGE-induced changes indicative of cellular oxidant stress was blocked by inclusion of antioxidants in the medium (probucol or *N*-acetylcysteine) or by prevention of access of AGEs to the cell surface with antibodies to either RAGE or LF-L. Similar results concerning AGE-induced oxidant stress were observed in MPs. To extend these observations to the in vivo setting, AGEs were infused into rodents and evidence of oxidant stress was monitored.<sup>21</sup> Immunostaining of lung tissue from animals infused with AGE albumin demonstrated the presence of oxidation-specific lipid-protein adducts, malondialdehyde epitopes,<sup>46</sup> in the vessel wall (Fig 4A) that were not observed in control animals infused with native albumin (Fig 4B). Note that controls with nonimmune IgG in place of anti-MDA IgG showed no staining (Fig 4C). In addition to EC staining, there was a more prominent association of malondialdehyde epitopes with smooth muscle cells, suggesting that they too are targets of AGE-induced oxidant stress when the glycated proteins are initially present in the intravascular space. Normal mice infused with AGE albumin displayed evidence of oxidant stress in a range of organs<sup>21</sup>: (1) levels of thiobarbituric acid-reactive substances were increased, (2) induction of heme oxygenase mRNA was observed, and (3) activation of NF- $\kappa$ B occurred (Fig 5A). Electrophoretic mobility shift assays with radiolabeled probe for NF- $\kappa$ B and nuclear extracts from livers of mice infused with AGE albumin showed the appearance of DNA binding activity (Fig 5A, lanes 2 and 7), which was not observed in control animals infused with native albumin (Fig 5A, lane 1). Addition of excess unlabeled NF- $\kappa$ B probe blocked the appearance of the AGE-induced band (Fig 5A, lane 8), but inclusion of an unrelated oligonucleotide for Sp1 was without effect (Fig 5A, lane 9). The induced gel shift band was a consequence of AGE interaction with cellular receptors, since antibody to either RAGE or LF-L blocked its appearance (Fig 5A, lanes 3 and 4, respectively) as well as a consequence of RAGE-mediated oxidant stress, since probucol blocked the appearance of the AGE-induced gel shift band (Fig 5A, lane 5).

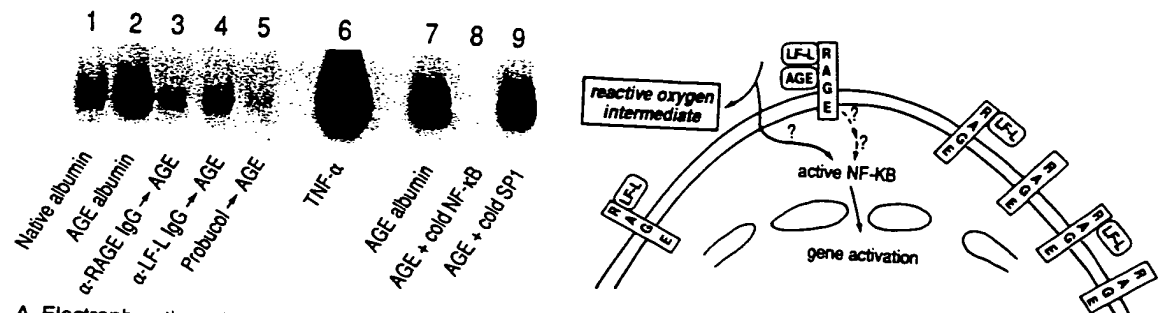


FIG 5. A, Electrophoretic mobility shift assay showing activation of NF- $\kappa$ B in mice infused with advanced glycation end product (AGE) albumin. Mice were infused with either AGE albumin or native albumin, livers were harvested, and nuclear extracts were prepared. Where indicated, mice were pretreated with either the antibodies or antioxidants as noted in the figure. The binding of nuclear proteins to the NF- $\kappa$ B site was demonstrated by electrophoretic mobility shift assay. All lanes contained the labeled probe, and other additions are indicated below the numbered lane. Reprinted with permission.<sup>21</sup> TNF- $\alpha$  indicates tumor necrosis factor- $\alpha$ . B, Schematic of AGE activation of the transcription factor NF- $\kappa$ B, as has been postulated in other situations of cellular oxidant stress.<sup>45</sup> RAGE indicates receptor for AGE; LF-L, lactoferrin-like polypeptide.

These data are consistent with the concept that AGEs, after interaction with their cellular receptors, are responsible for induction of oxidant stress and activation of NF- $\kappa$ B both in vitro and in vivo. The potential significance of NF- $\kappa$ B activation in the setting of diabetic vascular disease is emphasized by recent data linking this nuclear factor to transcriptional activation of cytokines and cell adherence molecules believed to have a central role in atherogenesis.<sup>47</sup> In this context, the interaction of AGEs with endothelium induces expression of vascular cell adhesion molecule-1 (VCAM-1),<sup>48</sup> an adherence receptor whose expression is one of the earliest events observed in experimental atherosclerosis.<sup>49</sup> AGE augmentation of VCAM-1 expression occurs as a consequence of the binding of AGEs to their cellular receptors, generation of oxidant stress, and activation of NF- $\kappa$ B. This results in enhanced adhesivity of the ECs for monocytes and could contribute to the accelerated and diffuse atherogenesis observed in diabetes.

Another setting in which generation of reactive oxygen intermediates by AGEs can exert far-reaching effects on cellular properties occurs when nonenzymatic glycation modifies long-lived intracellular proteins. An example of this phenomenon, emphasizing the potential of AGEs to contribute to a range of disorders, is oxidant stress associated with AGE-modified, paired helical filament tau in Alzheimer's disease. Neurons containing AGEs also displayed evidence of oxidant stress (by the same criteria as above<sup>28</sup>). AGEs that form intracellularly, especially on structures that have a slow turnover, can generate reactive oxygen intermediates within the cell, modulating cellular functions in a sustained fashion and rendering their interception by cellular antioxidant mechanisms less likely.

### Diabetic Red Cells and Endothelium

Studies of nonenzymatic glycation of diabetic RBCs have focused largely on hemoglobin.<sup>8,50</sup> In view of the long survival of RBCs in the circulation, we considered whether components of the cell membrane could also undergo nonenzymatic glycation, resulting in formation of AGEs and the capacity for enhanced interaction of diabetic RBCs with vascular cells expressing RAGE. Consistent with this hypothesis was the observation that

diabetic RBCs were reported to display increased adhesivity for ECs.<sup>51</sup> Our experiments have shown that preincubation of patient-derived diabetic RBCs with anti-AGE IgG or preincubation of ECs with anti-RAGE IgG blocks the enhanced binding of diabetic RBCs to the endothelium.<sup>52</sup> Infusion of diabetic rat RBCs into normal rats resulted in their accelerated clearance (compared with RBCs from normal rats), which was blocked in large part by anti-RAGE IgG.<sup>52</sup> These data suggest that diabetic RBCs exhibit enhanced interaction with RAGE expressed by cells at the vascular interface in vivo. We believe that the major pathophysiological significance of the enhanced interaction of diabetic RBCs with the vessel wall is the resulting induction of oxidant stress; this was observed both in vitro and in vivo after infusion of diabetic but not normal RBCs and would be expected to be greatly magnified in the microvasculature, where RBC-vessel wall interactions are frequent.

### Hypothesis

These data lead us to hypothesize that the interaction of AGEs with ECs and MPs, and probably multiple other cell types as well, involves RAGE and LF-L (Fig 5B). The integral membrane protein RAGE can bind AGEs alone or after formation of a complex with LF-L. Endothelial processing of AGEs in the intravascular space starts with cellular uptake and is followed in part by transcytosis with delivery and release of the glycosylated ligand to the subendothelial space. In addition, the interaction of AGEs with their receptors results in cellular activation, probably through multiple pathways. One mechanism is the induction of cellular oxidant stress caused by tethering of an oxidizing stimulus, AGE, by RAGE or RAGE/LF-L at the cell surface, resulting in activation of NF- $\kappa$ B. The potential impact of sustained generation of reactive oxygen intermediates by AGEs in diabetes is underscored by the recognition that diabetes is often accompanied by suboptimal function of protective antioxidant mechanisms.<sup>53-60</sup> In addition to generation of oxygen free radicals, other types of signal transduction are also likely to occur when AGEs interact with cellular receptors, and we are exploring these on the basis of the hypothesis that ligand-receptor interaction will recruit intracellular sig-

and transduction elements through interaction with the cytosolic tail.

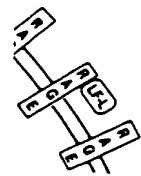
The presence of RAGE on ECs, MPs, smooth muscle cells, mesangial cells, and neurons suggests a potentially important role in the genesis of diabetic complications. In addition, interaction of AGEs with their receptors on ECs, smooth muscle, and MPs suggests a role in the pathogenesis of vascular disease in diabetes and atherosclerosis. This hypothesis lends itself to rigorous testing. First, studies concerning the regulation of RAGE expression and further delineation of the consequences of ligand-receptor interaction for modulation of cellular properties will have to be done both in vitro and in vivo. Then, the effect of receptor blockade on end-organ damage in diabetes will have to be examined by use of reagents that selectively block AGE-RAGE interaction and of genetically deletion mice. Understanding the contribution of AGEs and their cellular interactions to other disorders, such as Alzheimer's disease and hemodialysis-associated amyloidosis, should also be fruitful in terms of providing insights into disease mechanisms.

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end product (AGE) was prepared of nuclear proteins and other additions. Schematic of AGE formation occurs, leading to AGE. RAGE indicates

increased adhesion shown that RBCs with AGEs with anti-AGE at the vascular major pathologic interaction of the resulting RBCs but not RBC-vessel

interaction by multiple LF-L (Fig 3) can bind with LF-L. In part, the glycosylated results in pathways. oxidant stimulus, impact intermediate recognition. In other words, we are that is that



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